

Serial No.: 10/600,997  
Filing Date: June 20, 2003

**Amendments to the Specification:**

**Please replace the paragraph beginning at page 14, line 9 with the following rewritten paragraph:**

-- Figure 30 shows the BT LA allelic variation between a number of mouse strains (SEQ ID NOS:11-27).  
--

**Please replace the paragraph beginning at page 67, line 3 with the following rewritten paragraph:**

-- **Plasmid constructions.** Myc-tagged BT LA constructs were prepared as follows. The open reading frame of mBT LAs was amplified from a colony obtained from screening a DO11.10 TH1 cDNA library with primers J10-RV1-Bgl2 (5'-AGCTCTGAAGATCTCTAGGGAGGAAG-3') (SEQ ID NO:28) and J10-Xho1 (5'.-CATGCTCGAGGAAGGTCCAGACAGAGGTATTG-3'.) (SEQ ID NO:29). The product was digested with *Bg*/II and *Xho*I and cloned into the IRES-GFP-RV retrovirus48 at the *Bg*/II and *Xho*I sites to produce mBT LAs-RV. The N-terminal Myc-tagged version of mBT LAs (Myc3-mBT LAs-RV) contains a triple Myc tag inserted downstream of the signal peptide. To produce this construct, a PCR product containing the mBT LA signal sequence and 3. overhang homologous to the Myc tag was prepared with mBT LAs-RV as the template and primers J10-RV1-Bgl2 and J10-A2 (5.GTTCAGATCCAAGGATGCTCCAGAGGCC-3.) (SEQ ID NO:30). This PCR product was annealed to a second PCR product comprising three copies of the Myc epitope with 5. and 3. overhangs homologous to the N- and C-terminal portions of BT LA, respectively, which had been amplified from the triple Myc/Bluescript template with primers J10- A3 (5.-GAGCATCCTTGGATCTGAACAAAAGCTGATTA-3.) (SEQ ID NO:31) and J10-A4 (5.-CTTTCTCACAGAGCTCGTACAGGT CCTCT-3.) (SEQ ID NO:32). The triple Myc/Bluescript template contains 'anchor' sequences 5. (GS) and 3. (YEL) to the Myc3 coding sequence, which are included in the final Myc-tagged mBT LA protein. We then amplified the two annealed pieces with primers J10-RV1-Bgl2 and J10-A4. This product was annealed to a third PCR product containing a 5. Myc homologous tail and the C-terminal portion of BT LA amplified from the template mBT LAs- RV with primers J10-A5 (5.-GTACGAGCTCTGTGAGAAAGCTACTAAGAGG- 3.) (SEQ ID NO:33) and J10-Xho1, and the full-length chimeric cDNA was amplified with primers J10-RV1-Bgl2 and J10 Xho1. The resulting product was digested with *Bg*/II and *Xho*I and ligated into the *Bg*/II and *Xho*I sites of IRES-GFP-RV to yield Myc3-mBT LAs-RV. --

**Please replace the paragraph beginning at page 67, line 26 with the following rewritten paragraph:**

Serial No.: 10/600,997  
Filing Date: June 20, 2003

-- To produce the N-terminal Myc-tagged version of mBTLA (Myc3-mBTLARV), primers J10-RV1-Bgl2 and J10-A4 were used to amplify the signal sequence linked to the triple Myc epitope from template Myc3-mBTLAs-RV. A second PCR product was amplified with primers J10-A5 and J10 Xho1 and the template mj11W1. The two PCR products were annealed and amplified with primers J10-RV1-Bgl2 and J10 Xho1, digested, and ligated into the retroviral vector to produce Myc3-mBTLA-RV. A further modification was made by using the Quick Change mutagenesis kit (Stratagene) to convert a cysteine downstream of the Myc tag to alanine to mimic more accurately the predicted signal sequence processing in which this cysteine would be removed (SignalP V2.0). .cyt-Myc3-mBTLA-RV was generated using Quick Change mutagenesis of Myc3-mBTLA-RV with the primers mj11 trunc top (5.-

TGATATTCCATAAAC CTGCCACTGAGCCAG-3.) (SEQ ID NO:34) and mj11 trunc bottom (5.- TGGCAGGTTTATG GAATATCAACCAGGTTAGTG-3.) (SEQ ID NO:35). mBTLA-Myc2-RV, which expresses mBTLA with two C-terminal Myc epitopes, was generated by 'splicing by overlap extension' (SOEing) together two PCR products (generated from primers J10-RV1-Bgl2 and 3. mj11 Myc tail (5.- GCTTTGTTCACTTCTCACAAATGGATGC-3.) (SEQ ID NO:36) with template mj11W1, and primers 5. mj11 Myc tail (5.-TGAGGAGTGAACAAAAGCTGATTAGCGAAG-3.) (SEQ ID NO:37) and new 3. Xho Myc tail (5.-CCGCTCGAGCTCCTACAGGTCTTC-3.) (SEQ ID NO:38) with template triple Myc/Bluescript) with primers J10-RV1-Bgl2 and new 3. Xho Myc tail and *Pfu* polymerase. After digestion with *Bgl*II and *Xho*I, the PCR product was ligated into the retroviral expression vector Tb-lym-GFP RV49, which had been digested with *Bgl*II and *Xho*I, to generate mBTLA-Myc2-RV. --

**Please replace the paragraph beginning at page 68, line 6 with the following rewritten paragraph:**

-- The N-terminal Myc-tagged version of hBTLA containing a triple Myc tag inserted downstream of the signal peptide (Myc3-hBTLA-RV) was prepared similarly. Three separate PCR products were generated using the following primers and templates: 5. Bgl2 hj11 (5'- GAAGATCTGCAGGAAATGAAGACATTGCCT-3'.) (SEQ ID NO:39) and 3. Myc/hj11 bottom (5'- TCAGCTTTGTTCCCCATGGATGTTCCAGATGTCC- 3') (SEQ ID NO:40) with hj11#14u; 5. hj11/Myc top (5.-CATCCATGGGGACAAAAGCTGATTAGCGAAGAG-3.) (SEQ ID NO:41) and 3. hj11/Myc bottom (5.-CACATGATTCTTCAGGTCTCTCGCTAACAGC-3.) (SEQ ID NO:42) with triple Myc/Bluescript; and 5. Myc/hj11 top (5.- GAGGACCTGAAAGAACATGTGATGTACAGCTTA-3.) (SEQ ID NO:43) and 3. Xho hj11 (5.- CCGCTCGAGTTGGAGTCAGAACAGACTAAC-3.) (SEQ ID NO:44) with hj11#14u. These PCR products were sequentially annealed and amplified, and cloned into tb-lym-GFP-RV, which had been digested with *Bgl*II and *Xho*I. hBTLA containing three carboxy-terminal Myc epitopes (hBTLA-Myc3-RV) was generated by SOEing together two PCR products (from primers 5. Bgl2 hj11 and 3. hj11 Myc

Serial No.: 10/600,997  
Filing Date: June 20, 2003

tail (5.-TGAGGAGTGAACAAAAGCTGATTAGCGAAG-3.) (SEQ ID NO:45) with template hJ11#14u, and primers 5. hJ11 Myc tail (5.-TGAGGAGTGAACAAAAGCTGATTAGCGAAG-3.) (SEQ ID NO:46) and new 3. Xho Myc tail with template triple Myc/Bluescript) with primers 5. Bgl2 hJ11 and new 3. Xho Myc tail and *Pfu* polymerase. After digestion with *Bg*/II and *Xba*I, the PCR product was ligated into retroviral expression vector Tb-lym-GFP-RV49, which had been digested with *Bg*/II and *Xba*I, to generate hBTLA-Myc3-RV. Embryonic stem cells (MC50) were a gift of R. Schreiber. --

**Please replace the paragraph beginning at page 68, line 25 with the following rewritten paragraph:**

-- **Tyrosine mutations.** Single tyrosine-to-phenylalanine mutations of hBTLAMyc3- RV were produced using Quick Change mutagenesis and *Pfu* polymerase (Stratagene) with the following oligonucleotide pairs: Y226F top2 (5.-GAAACTGGAATTATGATAATGACCCTGACCTTG-3.) (SEQ ID NO:47) and Y226F bot (5.-GGGTCATTATCAAAAATTCCAGTTCTGATAGCAG-3.) (SEQ ID NO:48); Y257F top2 (5.-ACCAGGCATTGTTATGCTCCCTGAACCATTCTG-3.) (SEQ ID NO:49) and Y257F bot (5.-AGGGAAGCAAAACAATGCCTGGTTGT-3.) (SEQ ID NO:50); Y282F top2 (5.-GCACCAACAGAACATGCATCCATATGTGTGAGG-3.) (SEQ ID NO:51) and Y282F bot (5.-ATATGGATGCAAATTCTGTTGGTGCTTCTTTA-3.) (SEQ ID NO:52). We produced double and triple tyrosine-to-phenylalanine mutations of hBTLA-Myc3-RV by using the oligonucleotide pair Y257F top2 and Y257F bot first with the Y226F-mutated hBTLA-Myc3-RV template to produce Y226F/Y257F and then with the Y282F-mutated template to produce Y257F/Y282F. The oligonucleotide pair Y282F top2 and Y282F bot was used with the Y226F-mutated template to produce Y226F/Y282F, and with the Y226F/Y257F-mutated template to produce Y226F/Y257F/Y282F. --

**Please replace the paragraph beginning at page 71, line 23 with the following rewritten paragraph:**

-- We used an EST (aa839766) expressed by Th1, but not Th2, cells to screen a Th1 cDNA phage library made in the Lambda ZAP vector (Stratagene) and isolated a partial clone, BTLAs, that lacked an Ig domain. Full length BTLA cDNA, amplified from WEHI cell RNA by RT-PCR with primers J10-3K (5'-TTTGGCTAAGATGCTGCTA-3') (SEQ ID NO:53) and J10-7F (5'-CACAGATTGGGTACGACATG-3'.) (SEQ ID NO:54), was inserted into the GEM-T Easy Vector (Promega) to produce mJ11W1. We obtained additional full-length BTLA cDNA isolates by screening a second mouse splenocyte cDNA library (Stratagene) using the 5. region of mJ11W1 as a probe. Coding sequence and intron-exon boundaries were further determined by sequencing 129SvEv strain bacterial artificial chromosome clones containing the BTLA region (Genome Systems). Some Ig domain sequence polymorphisms occur among mouse strains. Human BTLA cDNA, amplified from Ramos B lymphoma

Serial No.: 10/600,997  
Filing Date: June 20, 2003

RNA by RT-PCR with primers hJ10 (5'-TTTTCCATCACTGATATGTGCAGG-3') (SEQ ID NO:55) and hJ10 AS (5'-GGTCCCTGTGGAGTCAGAAC-3') (SEQ ID NO:56) based on the Celera human genome assembly, was inserted into the GEM-T Easy Vector to produce hJ11#14u. The Celera database sequence predicted the human BTLA amino acid sequence set forth in Figure 19 (SEQ ID NO:6), which differs from the BTLA sequence obtained from Ramos cells (SEQ ID NO:8) at amino acid residue 138. This is likely due to polymorphism, given the different human sources. The BTLA sequence as found in Ramos cells (SEQ ID NOs:7 and 8) was used for experiments disclosed herein. --

**Please insert the enclosed 20-page text entitled "SEQUENCE LISTING" immediately preceding the claims.**